**Life Extension Factor Klotho Enhances Cognition**
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**Introduction**
*This study investigates how the gene expression of the protein “Klotho” affects memory and lifespan (although, mostly memory).*

Klotho is a protein that can be released into the blood stream by cells (mostly attributed to cells of the kidney, but other cells in the body are capable, as well) and this release of klotho into the blood stream may have an impact on lifespan and memory. So, the idea is, if there is more klotho expressed, is this beneficial?

NMDAR: NMDA receptors; meaning, these receptors are located on neurons (brain cells) and they are activated by particular neurotransmitter glutamate, when it binds. Once activated, these neurons are excited/activated.

GluN2B: The NMDA receptor is made of a bunch of smaller proteins (called subunits) – GluN2B is one of those subunits (as with all other GluN2 proteins mentioned).

**Methods**
This is a hybrid human/animal/cell study.
 - Human participants with a Klotho mutation vs human participants without the mutation.
 - Genetically altered mice with the Klotho mutation vs mice without the Klotho mutation.

*Human Participants*:
 - There were 718 participants, in total.
 - Normal/Non-klotho mutation: 530 participants
 - Klotho mutation: 188 participants
 - All participants were between ages 52 and 85 years.

Mice Subjects:
 - Transgenic Klotho Mutants: Mice that overexpress/express more Klotho.
 - Non-transgenic Mice: Normal mice with normal expression of Klotho.

**Results**
*Figure 1A-D*
*Background*: This figure is part of the human data explained in the methods section of the notes. 718 total participants, split between three groups (1A-C) with 1D showing all the groups combined for the total, of which 530 are normal expressing klotho individuals (Non-Carrier) and 188 carry a klotho mutation (KL-VS) that leads to greater expression of the klotho gene and formulation of the protein for secretion. These graphs show the differences in scores on the “Mini-Mental State Exam” that measures the cognitive ability of middle and late age individuals.

*Primary Results*:
- Across all 4 panels (A-D), more Klotho overexpressing (KL-VS) individuals have a high score (over 28, which is considered great) on the cognitive test (1C has a p = 0.06, likely significant).

Take Away: More Klotho expression is correlated with better cognition/mental performance.

*Figure 2A-D*
*Background*: This figure shows the relationship between age and performance on that cognitive test mentioned in Figure 1, between the normal Klotho expressing individuals (Non-Carriers) and Klotho expressing individuals (KL-VS).

*Primary Results*:
- Klotho is still correlated, across all ages, with better cognitive outcomes (as measured by this test).
- Klotho individuals also see declines in cognitive performance with age, albeit not quite as severe as normal Klotho expressing individuals.

Take Away: More Klotho expression is related to a higher cognitive ability across all ages compared to normally expressing individuals, but does not protect against the effect of aging.

*Figure 3A*
*Background*: This graph shows the amount of circulating Klotho protein in the blood between normally expressing and overexpressing participants.

*Primary Results*:
- There is more Klotho circulating in Klotho overexpressing mutants (KL-VS).

Take Away: People with the Klotho mutation from the previously shown data do, indeed, have more Klotho protein circulating.

*Figure 3B*
*Background*: The experiments have shifted to mice; this quantifies the amount of Klotho protein found in the hippocampus (memory center of the brain) between normal mice (NTG) and Klotho overexpressing mice (KL).

*Primary Results*:
- There is greater Klotho protein expression in Klotho overexpressing mice, in the hippocampus.

Take Away: Klotho is higher, in the memory center of the brain, for Klotho overexpressing mice.

*Figure 3C*
*Background*: This experiment shows the lifespan difference between normal expressing Klotho mice (NTG) and Klotho overexpressing mice (KL).

*Primary Results*:
- Klotho overexpressing mice live longer than normal mice.

Take Away: This offers some proof that greater amounts of Klotho increase lifespan.

*Figure 3E*
*Background*: This experiment is one wherein the mice are taught where a plate is located in a bath (the only location in the bath where the mouse can stop swimming) and then removed, and then reintroduced to the bath to see how long it takes them to find the plate again. NTG = Normal mice. KL = Klotho overexpression mice.

*Primary Results*:
- Klotho overexpressing mice take significantly less time to find the plate.

Take Away: Klotho overexpressing mice remember/have better memory (presumably).

*Figure 5A&F*
*Background*: The researchers are measuring the amount of protein/molecule found in neural (brain) cells. The fractions are different sections of the cell, and the “+” symbol represents the presence of Klotho overexpression. GluN2A,B,C are all subunits of the NMDA receptor that activates/excites neurons (more details in the introduction section of these notes). Synaptophysin is simply a marker of the cell membrane and PSD is another marker of the cell – these are used to confirm that the researchers are specifically looking at the sections of the cell they want. 5A is in the hippocampus/memory section of the brain, and 5F is in the cortex/thinking (along with other tasks) section of the brain.

*Primary Results*:
- There is greater expression/amount of GluN2B subunit of the NMDA receptor without increases in the other two subunits, in both sections of the brain (hippocampus or cortex).

Take Away: Klotho overexpression seems to increase the amount of this particular subunit, but if this implies more NMDA receptors, greater activation, or anything else is unknown.

*Figure 6C*
*Background*: The researchers have taken brain slices and are stimulating them to record the excitatory/activating reaction of the neurons (brain cells), in 4 month old/adult normal mice and overexpressing Klotho mice, in the dentate gyrus (part of the hippocampus/memory section of the brain).

*Primary Results*:
- Klotho overexpressing mice neurons/brain cells have a greater ability to maintain excitation.

Take Away: Although this is not necessarily shown, the assumption is if the neurons can be excited more consistently, they will have a greater ability to form connections between them (long term potentiation).

*Additional Notes*
The researchers also acknowledge that there have been studies on homozygous (both Klotho genes overexpressing Klotho, instead of just one – as seen in this study) Klotho mutation leading to even higher Klotho levels and these have been widely considered \*detrimental\* to cognition and lifespan.

GluN2B subunit closes the NMDA receptor more slowly than the other subunits, so it could lead to more signaling/more activation time of the neurons.

**Conclusions**
Increased (up to a point) Klotho likely improves cognition, brain function, memory.

Increased (up to a point) Klotho may extend lifespan.

Both of these outcomes are largely mechanistically unknown; Klotho has an impact on NMDA receptors by increasing GluN2B amount, but beyond that, we don’t know how this fits into the grand scheme beyond potentially increasing the ability for neurons to be activated more robustly/consistently.